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THE PHYSIOLOGICAL ASPECTS, TECHNIQUE AND MONITORING OF SLAUGHTER PROCEDURES AND THEIR EFFECTS ON MEAT QUALITY – A REVIEW

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Abstract

The aim of the paper was a review of the scientific achievements in physiological stunning and slaughtering mechanisms, control methods of consciousness and their effect on meat quality. Special attention was paid to neurophysiological phenomena that accompany the process of depriving consciousness before animal deaths using mechanical, electrical and gas stunning methods. These mechanisms are associated with cerebral hypoxia or ischemia or depolarization, acidification and the destruction of cerebral neurons. Such effects can be caused by shock waves, bleeding, electric fields, reduction or arrest of the circulation of blood in the brain, high CO_2 level or low O_2 level in inhaled air or by the mechanical damage of neurons. Some of the stunning methods cause immediate and some gradual consciousness loss. An important factor in the animals' slaughtering process is the estimation of their consciousness level before bleeding. The indicators of consciousness during mechanical, electrical and gas stunning are discussed within this paper. It is pointed out that at least 2 indicators should be used when estimating animals' consciousness after stunning, e.g. phonic and clonic limb movements and lack of breathing. Ten indicators to control the consciousness were described. The effect of stunning on meat quality is also discussed. It was found that the impact of this process on the quality is not clear. However, the prevailing view is that electric stunning causes effusions and blood haemorrhages in meat. Whereas gas stunning with a CO_2 mixture diminishes the risk of PSE meat. Despite numerous scientific research on the slaughter process there is still deficiency in knowledge on losing consciousness mechanisms and feeling pain. It might be useful to extend the knowledge concerning neurotransmitters and use of magnetic resonance in future studies.

Key words: stunning, consciousness, unconsciousness indicators, bleeding, meat quality

The basic condition of the humanitarian slaughtering of animals and their welfare during the process is the loss of consciousness during slaughtering. Animals have been slaughtered by people since time immemorial. Within the last couple of decades special attention has been paid to minimizing the pain and suffering to animals

during slaughter. Based on the suspicion that loss of consciousness after electric stunning together with bleeding was a humanitarian slaughtering method it became one of the most commonly used methods of slaughtering almost all animal species (Zivotofsky and Strous, 2012). Gas stunning with carbon dioxide and mechanical stunning with a radical gun as well as ritual slaughtering without stunning are also popular methods (Gregory, 2005; Terlouw et al., 2016 a). It is impossible to decide which method causes the least pain without recognizing the neurobiological and physiological mechanisms of stunning and slaughtering as well as without proper methods of estimating the effectiveness of pain elimination during slaughter (Terlouw et al., 2016 b; EFSA AHAW Panel, 2013). Apart from the above issues the most important are the techniques of stunning and its effect on meat quality. These aspects, associated with meat defects such as blood haemorrhages and PSE meat, are very important for economical effectiveness of slaughterhouses (Velarde, 2000; Kien, 1999).

The aim of the paper is a review of the scientific achievements in physiological stunning and slaughtering mechanisms, control methods of consciousness and their effect on meat quality.

Legal regulations and general physiological aspects of stunning and slaughtering

The basic EU law regulation concerning the animal slaughter is the EC Regulation No. 1099/2009 of 24th September 2009. The law settles the rules concerning the killing of farm animals bred and reared for food, wool, leather, furs or other products. It also settles the rules of killing animals in order to lower their number. Some definitions included in the regulation are worth mentioning. The killing of an animal is a number of intentional activities causing its death. The killing activities are as follows: animal treatment before killing, storing, cramping, stunning and bleeding performed in order to kill animals at the place where they should be slaughtered. The stunning is a number of intentional activities causing painless loss of consciousness and loss of sensitivity to stimuli including number of activities causing instant death. Animal slaughter means killing of animals intended for human consumption. It is stated in paragraph 4 that animals shall be killed only after stunning performed according to the methods and precise requirements described in the annex. The list of methods and chosen requirements are described in the further sections within this paper.

In order to understand the basic processes of losing consciousness while using proper slaughter techniques it is necessary to recognize the role of different brain structures within the process. Consciousness is a complex concept embracing two states: the sense state and the consciousness state (Terlouw et al., 2016 a). Pain might be caused by tissue damage or by mechanical, chemical or thermal tissue stimulation. This negative impulse is sent to the brain reaching its different structures e.g. cerebral hemispheres, thalamus, pons, medulla oblongata, midbrain, cerebellum, and hypophysis (Figure 1). Pain is connected with negative feelings and is regarded as a potential stress factor. It is regarded that the structures of the brain cortex do not function in the state of lost consciousness and there is no feeling of pain. An excep-

tion are scientific reports that proved some residual pain felt in the state of unconsciousness (Chatelle et al., 2014).

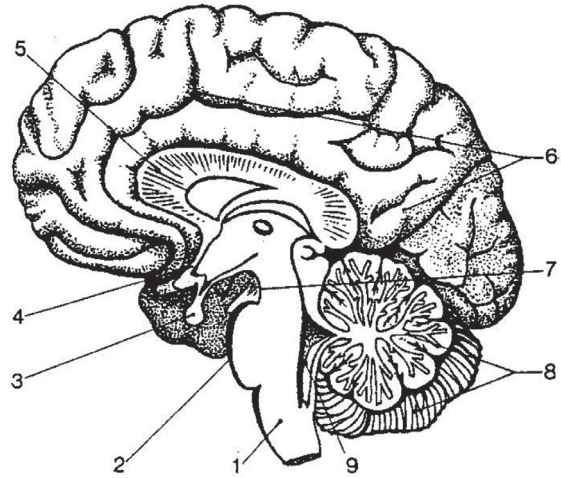

Figure 1. Cerebral structures bound with pain and stunning of animal: 1 – medulla, 2 – pons, 3 – hypophysis, 4 – hypothalamus, 5 – corpus callosum, 6 – cerebral hemisphere, 7 – midbrain, 8 – cerebellum, 9 – ventriculus quartus encephali (Praca zbiorowa, 2000)

Pain is the source of physical stress whereas fear is a type of psychological background stress (Terlouw et al., 2008). Fear is a mechanism that lets animals identify and avoid danger and increases the chance of survival. Fear might cause defence reactions (attack, threat), escape or immobility. Behavioural reactions to pain and fear are accompanied by classic, physiological reactions such as increased heart beat, higher catecholamine and glucocorticoids levels in blood plasma (Boissy et al., 2007).

Stunning before bleeding must cause the state of unconsciousness in slaughtered animals. Unconsciousness might be reached by causing a dysfunction of brain hemispheres, reticular formation or thalamus. Cattle in Europe are usually stunned mechanically using a bolt gunshot causing a shockwave with partial, mechanical brain damage. Electric stunning is most commonly used in other species (pigs, sheep, calves and poultry). The electric current of defined parameters running through the brain (see subsection 3.2) is key in this method. The electric current causes the massive depolarization of neurons in both cerebral hemispheres. Gas stunning with a high concentration of CO_2 is becoming more and more popular in pig and poultry slaughterhouses. Carbon dioxide dissolves in the blood after being inhaled and enters the brain causing hypoxia leading to loss of consciousness. All these stunning techniques cause the improper functioning of brain neurons and lead to a reversible or irreversible state of unconsciousness depending on the technique used and its parameters.

Techniques and physiological aspects of different stunning methods

Mechanical stunning

According to the regulations, mechanical stunning, including bolt stunning, can be used in all animal species (Council Regulation, 2009). However, they are most commonly used in cattle and horses (Final Report, 2017). The aim of the bolt technique is to damage the brain structure responsible for the senses i.e. with reticular formation (Finnie et al., 2002). The effect of this technique is a strike in the skull with a bolt shank to send the bolt into the brain. The strike causes a shockwave in the brain and brain tissue damage as well as disruption of blood flow (Posner et al., 2008). It also causes rapid potassium leakage from the cells causing the depolarization of cerebral hemispheres as well as the secretion of neurotransmitters and calcium leakage from cells, mitochondria damage and energy release in the cells. These disorders stop any regular neuron functions. The bleeding deprives some brain structures of blood and as a consequence less and less oxygen and nutrients stay inside and outside the cells which is crucial for biochemical balance. Parts of the skull that enter the brain after the shot was fired also cause further brain damage. The research shows (Terlouw et al., 2016 a) that the bolt damages the brain hemispheres, cerebellum and midbrain and in many cases also pons and the medulla oblongata (Figure 2). It is recommended to put the end of the bolt gun in the centre of the forehead and especially:

– in cattle 3–5 cm above the cross of two lines running from the ear to the eye (Terlouw et al., 2016 a),

– in horses the target is a point 1–2 cm above two lines cross running from the inner corner of each eye to the upper edge of the attachment of the opposite ear (Final Report, 2017),

– in sheep without horns right above the eye line; in sheep with horns on top of the head (Terlouw et al., 2016 a),

– in pigs in the middle of the forehead, 2.5 cm above the eyebrows (Grandin, 2013),

– in rabbits place the stunner firmly against the rabbit's head on the midline and at the intersection of lines drawn from the outside edge of the eye to the base of the opposite ear (Final Report, 2017).

Apart from bolt stunning the mechanical method based on the hit in the head is also allowed (EC Regulation, 2009). The hit should cause serious brain damage but it can only be applied to piglets, lambs, goatlings, rabbits, hares, fur animals and lightweight poultry (up to 5 kg live weight). This method however cannot be applied as a routine procedure, it should only be used when there are no other stunning methods available.

In the case when after shooting damage to the brain has not reached the respective brain structures and when the animal is partially or fully conscious, then another shot is required immediately (Bourguet et al., 2011). The proper use of a radical bolt gun immediately stops the breathing but the heart continues to beat for 8 to 10 minutes if the bleeding is not completed sooner (Finnie et al., 2002). The head of the radical can be placed in the wrong position due to different brain localization with regard to the forehead depending on the cattle breed or sex (Gregory et

al., 2007). Mistakes in the stunning process occur more often in bulls because their skulls are thicker and bigger.

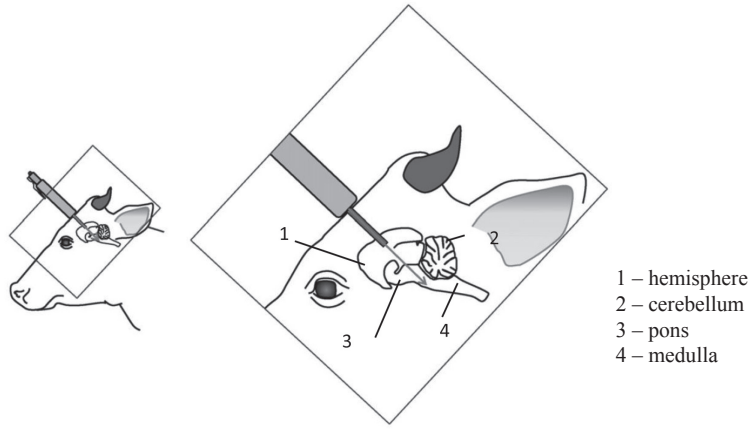

Figure 2. Correct placement of captive bolt pistol, targeting the pons of the brainstem (Terlouw et al., 2016 a)

Electrical stunning

According to the regulations, electrical stunning can be used in all animal species. However, it is most commonly used in the slaughter of pigs, sheep and poultry (Council Regulation, 2009).

Electric stunning causes dysfunction in brain activity by putting electrodes on both sides of the head and then the electric current flows through brain damaging its regular electrical activity (Terlouw et al., 2016 a). The lost electric field leads to depolarization of the cell membranes in many neurons and causes an epileptic seizure (Blumenfeld, 2005). The seizure might cause shorter or longer consciousness loss. If the seizure embraces not only the cerebral cortex, the thalamus and brain stem but also the under cortex brain structures, then it is called a general seizure that causes deep unconsciousness. At the moment a seizure occurs the brain needs oxygen which is 2 to 3 times higher than at a normal physiological state and the demand for blood is lower at the same time. The level of lactic acid goes up and some of the brain structures are acidified. An epileptic seizure also causes a higher concentration of neurotransmitters (pain indicators) that plays a crucial role in the ions that flow through the neuron cells membranes. All these dysfunctions, if strong enough, cause loss of consciousness as well as convulsions, hyperactivity of the peripheral nervous system and hyper secretion of endocrine glands (Blumenfeld and Taylor, 2013).

There are two types of electric stunning used in practice: the two electrodes type and the three electrodes type (Final Report, 2017). The two electrodes one (head only) is based on putting the electrodes on both sides of animals' head so that the electric current can flow through the brain. When using proper electric current parameters unconsciousness happens immediately but it is reversible. This method is used on pigs, sheep, poultry and in some countries also on cattle. The three elec-

trodes stunning is called the "head-body" method and is used on pigs, sheep, cattle and poultry. The electrodes are put in the following order: two on the head and the third electrode is put on the sternum, close to the heart (Weaver and Wotton, 2009).

The parameters of the electric current used for stunning i.e. voltage, time and frequency are selected depending on the species and method of stunning. The use of a net frequency of 50-60 HZ causes heart fibrillation that hinders proper blood circulation. Brain hypoxia occurs which deepens the unconsciousness. Very often the heart fibrillation stops the heart ad the animal is dying before bleeding (Vogel et al., 2011).

The EU regulations determine the following minimum electricity values for electric stunning method called "head only" (Council Regulation, 2009):

– cattle at the age over 6 months: 1.28A,
– cattle at the age up to 6 months: 1.25A,
– sheep and goats: 1.00A,
– pigs: 1.30A.

In case of pigs the European Commission expands the recommendation concerning the "head only" stunning methods (Final Report, 2017) and it is presented in Table 1.

Table 1. Recommended parameters for head-only electrical stunning of pigs

Category	Minimum voltage	Minimum amperage	Frequency	Minimum duration
Adult pigs	> 250V	1.30A 1.8 A (if > 150 kg) 2A (sows and boars)	50–800 Hz	1–8 sec
Piglets	> 250V	1.30 A	50–800 Hz	5–8 sec

For automatic systems, some use high voltages (600 to 1000 V) for 3 seconds. As a result, most pigs are killed and only few are stunned. Other automatic stunners use a high current (for example, from 1.8A to 2.3 A), which can be combined with a shorter duration (for example 2 seconds).

Table 2. Requirements for electric amperage in devices for stunning poultry in a water bath – minimum values (Council Regulation, 2009)

Frequency	Chickens	Turkeys	Ducks and Geese	Quails
< 200 Hz	100 mA	250 mA	130 mA	45 mA
From 200 to 400 Hz	150 mA	400 mA	Not Allowed	Not Allowed
From 400 to 1 500 Hz	200 mA	400 mA	Not Allowed	Not Allowed

In case of "head body" stunning method the minimum electricity required for sheep and goats is 1 A and 1.3A for pigs. The regulation does not settle other parameters for the "head-body" stunning. When it comes to poultry stunning in the water bath the regulation includes not only the electric current strength but also its

frequency and time it should be applied, i.e. at least 4 seconds (Table 2). The chicken stunning with frequency of 50 Hz or less and electric current of 120 mA is regarded as a more effective stunning method (Final Report, 20017).

"Head only" electric stunning method is regarded as "the good practice" and is also recommended in rabbits stunning, especially in the plants of higher capacity (Final Report, 2017). The following electric current parameters are required in this method: voltage 100–117 V, amperage 140–400 mA, minimum stunning time 0.5–3 sec. The stunning phase in rabbits is not long so the bleeding should follow as fast as possible.

There were some trials to stun animals with microwaves causing rapid brain temperature increase and not resulting in widespread use (McLean et al., 2017). But in order to obtain a 20°C increase in brain temperature using 434 MHz frequency waves it is required to use a generator with 45–70 kW power. Such a requirement is not possible to be fulfilled in daily practice. Further trials are being performed in order to improve this method as it might be applied in ritual slaughter (reversible stunning). In this method, frequency was tuned to 922 MHz and the generator power was decreased to 9 kW and a positive effect in cattle stunning was observed (McLean et al., 2017).

Gas stunning

Stunning with carbon dioxide is performed by placing the animal in a gas chamber filled with an oxygen mixture of proper CO_2 concentration. This method is used on pigs and poultry. A group of pigs enters the gondola that lowers to the shaft filled with the CO_2 mixture of at least an 80% concentration as recommended (Council Regulation, 2009). The gas stunning in poultry slaughtering with carbon dioxide is done in two steps. First is the exposure of the conscious bird to the gas mixture containing 40% CO_2, and after losing consciousness the higher gas concentration is applied.

While alive the regulation of CO_2 and O_2 concentration in the animal's blood is running properly. Oxygen is crucial for an animal organism's energetic metabolism whereas carbon dioxide is a metabolite that must be removed from the organism. This process runs in the pulmonary alveoli where the gas molecules move from the places of higher concentration to places with a lower concentration. When the carbon dioxide concentration is higher in the inhaled air the gas is absorbed by the blood and delivered to the brain causing hypoxia and acidification of the nervous cells. It is followed by brain activity depression leading to sense and consciousness loss or even to death (Rodriguez et al., 2008). The carbon dioxide content regulation in blood is a key factor to the pH regulation. Only 5% of CO_2 is diluted in blood. The majority of the carbon dioxide is transported by blood in HCO_3^- ions. The protons are released and haemoglobin is their buffer. When the haemoglobin buffer capacity is exceeded the cerebrospinal fluid is acidified as a result of high CO_2 concentration and low blood pH. All these circumstances lead to unconsciousness (Table 3).

The time needed to reach unconsciousness is shorter when the CO_2 concentration in the shaft is higher. The pigs in the study fell down after 38, 34, 25, 17, 22 and 15 seconds of exposure to CO_2 at the concentration levels 40, 50, 60, 70, 80 and

90% respectively (Raj and Gregory, 1996). The research of Martoft et al. (2002) showed a lack of brain reaction in pigs after 21 and 15 seconds of inhaling the air containing 80% and 90% of CO_2 respectively. The gas stunning has a negative effect on pigs' welfare to some point. The breathing changes within a couple of seconds after dipping in the CO_2 mixture. Breathing is faster and deeper, convulsions appear and behaviour is weaker and less controlled. Inhaling CO_2 in animals causes pain and sensational behaviour which increases with higher gas concentration (Gregory, 2005). Consciousness is lost on average after 60 seconds from the beginning of stunning whereas convulsions occur after 19 seconds (Rodriguez et al., 2008). Different concentration of CO_2 in "Compact" device was used. In this method the concentration of CO_2 in the first phase is 35% during 15 s, then increased to 70–75% during 30 s and at the end of the stunning process during 15 s decreased to 35%. After stunning the animals are in deep anaesthesia (full unconsciousness) during next 30–60 s (Chwastowska-Siwiecka and Baryczka, 2019).

Table 3. Influence of gas stunning on blood parameters in pigs (Terlouw et al., 2016 a)

References and CO_2 concentration	Time of CO_2 exposure	Arterial PO_2 (mm Hg)	Arterial PCO_2 (mm Hg)	Arterial pH
Forslid and Augustinsson, 1988: 80% CO_2	Before exposure	102.6±3.0	40.3±1.5	7.45±0.01
	After 15 s	55.5±6.8	308.6±28.9	6.74±0.03
	After 45 s	29.6±1.5	397.5±25.1	6.63±0.01
Martoft et al., 2003: 90% CO_2	Before exposure	100±1%	34.2±7.6	7.57±0.02; (brain: 7.28±0.14)
	After 58 s of exposure			(brain: 6.73±0.13)
	15 s after the end of the exposure	53±1%	282.0±7.6	6.78±0.02
Rodríguez et al., 2008: 90% CO_2	Before exposure	79.0±8.2	43.0±5.5	7.42±0.04
	After 15 s of exposure	37.5±7.5	447.0±122.2	6.71±0.12

The EU regulation allows using carbon monoxide in poultry, pigs and fur animals stunning (Council Regulation, 2009). Conscious animals are placed in the hermetically isolated chamber containing gas mixture with more than 4% of carbon monoxide. The animals enter the chamber individually and stay there until they are dead. This method is almost painless but its main disadvantage is lack of safety because carbon dioxide is deadly dangerous for people. Carbon monoxide is odourless and colourless and is impossible to be detected with human senses (Praca zbiorowa, 2000). It adjoins extremely easy to haemoglobin forming carboxyhaemoglobin that blocks oxygen transportation from the bloodstream to the cells causing their death by hypoxia. Even at the low levels of carboxyhaemoglobin, e.g. 5% in the human blood the organism shows disorders like inability to recognise colours, at the level of 10% the headache appears. Brain and heart are the most sensitive organs to lack of oxygen and they get damaged first.

Some of the gas stunning systems are based on immersion in neutral gases such as nitrogen or argon (Terlouw, 2016 a) and can be used in slaughtering pigs and poultry (Council Regulation, 2009). The method is based on low oxygen concentration. Lack of oxygen in the air mixture leads to brain hypoxia resulting in stunning. Such systems are used in some poultry slaughterhouses and only experimentally in pigs because there are no proper technical constructions for this species.

Descriptions of consciousness and unconsciousness indicators

The European Food Safety Authority expert team presented in 2013 in Parma their scientific opinion on monitoring procedures for animals in slaughterhouses. In the opinion ten indicators of pig's consciousness have been defined, as described below, and most of them concern other species of animals (EFSA Journal, 2013).

Breathing

Effective electrical stunning should cause immediate apnoea. Ineffectively stunned animals regain consciousness and start breathing and the rhythmic inhaling and exhaling is clearly visible by chest and nose movements. If the movements are not as clear the breathing might be recognized by putting a mirror next to the animals' nose because humid exhaled air is condensed as steam on a mirror surface.

Corneal reflex

Corneal reflex is observed after touching or lightly striking the cornea of an eye. Insufficiently stunned animals regaining consciousness will blink in a reaction to such stimulation.

Eye movements

Fixed, widely open and "glass" eyes with clearly visible iris/cornea are indicators of unconsciousness. In some stunned animals the eye balls may be seen moving around the eye sockets. In insufficiently stunned animals regaining consciousness the eye movements are normal.

Muscle tone

Animals stunned with the "head only" electric method show a lack of muscle tone after the tonic-clonic limbs, tail, jaws and tongue movements have stopped. Animals insufficiently stunned show regular muscle reflexes and trials to stimulate the heart.

Palpebral reflex

This reflex occurs after touching or striking the eye with the finger. Properly stunned animals do not show the eyelid reflex whereas improperly stunned animals will blink.

Posture

Effective electric stunning causes rapid fall down and animals lose their standing position. Improperly stunned animals do not fall or try to get up after falling down.

Nose prick or ear pinch

The reaction to pain stimulation by the nose prick or ear pinch proves that animals are still conscious after electric stunning.

Spontaneous blinking

Conscious animals show spontaneous blinking whereas unconscious animals do not blink.

Tonic seizures

Effective electric stunning leads to tonic-clonic limb movements right after falling down. Tonic movements as tetanic movements last for a couple of seconds and are followed by clonic movements leading to loss of muscle tone.

Vocalisations

Conscious animals may react with sound to pain. That is why vocalisation is used to estimate the effectiveness of stunning. It should be underlined that not all conscious animals may vocalise.

The consciousness control indicators for animals stunned electrically

The estimation of animals consciousness stunned with manual electric electrodes should be performed in 3 stages (EFSA Journal, 2013). The first stage is between the end of stunning and hanging the carcass on the hook. The tonic-clonic limb movements are estimated together with breathing and corneal or palpebral reflex. Additionally the following indicators might be used: blinking, posture and vocalisation. The second stage is the carcass at the moment of sticking when indicators like: breathing control, tonic-clonic limb movements, and muscle tone are estimated. Additionally the corneal reflex or palpebral reflex as well as the spontaneous blinking might be checked. The third stage i.e. while bleeding the breathing and muscle tone is controlled and additionally the corneal reflex or palpebral reflex as well as the spontaneous blinking might be checked.

The consciousness control indicators for animals stunned with carbon dioxide

The EFSA team suggestions regarding the 3 stage control system are also applied in gas stunning. The effectiveness of stunning should be estimated at each stage at least with 2 indicators. In the first stage it is recommended to estimate muscle tone, breathing and corneal reflex or palpebral reflex. Additionally it is recommended to estimate vocalisation and reaction to the nose prick or ear pinch. In the third stage muscle tone and breathing and additionally the corneal reflex or palpebral reflex as well as vocalisation should be controlled.

Opinions on the effectiveness of the animal stunning process and its impact on the quality of fresh pork and ruminant's meat

According to experts further progress in this field is necessary despite the obligatory procedures of consciousness estimation in the slaughter process (Terlouw et. al., 2016). Further research on the interpretation of the consciousness control indicators and their correlation with different types of brain damage is needed (EFSA Journal, 2013). Thanks to this knowledge the risk of a wrong diagnosis of stunning efficacy could be minimized. It is necessary to broaden the knowledge in other objective welfare indicators in the stunning process like glucose, lactic acid concentration, settling the haematocrit and blood temperature while bleeding (Brandt and Aaslyng, 2015).

There are some opinions that electric stunning does not completely eliminate pain and thus the method is not fully satisfactory (Zivotofsky and Strous, 2012). The ETC (electroconvulsive therapy) method used in psychiatry when applied in the slaughtering of animals for electric stunning proved that incorrectly stunned animals or even animals stunned correctly with properly placed electrodes and with proper electric current parameters, still feel pain caused by sub-convulsive stimulation. Due to that fact such slaughtering is regarded as less humane than slaughtering without stunning. Further research on electric stunning parameters needs to be performed in order to define their optimal values which is crucial on a slaughter line especially when part of the slaughtered animals require second stunning as they were not stunned properly the first time, e.g. in Great Britain it is 15.6% of slaughtered pigs (Brandt and Aaslyng, 2015).

Albeit regulations recommend stunning electrically only once it is very common in practice to repeat the electric stunning twice in the manual method "head only". It happens when the first stunning is ineffective because of too short an application or incorrect positioning of the animal next to the employee at the moment of putting electrodes into the head or due to the animal's aggressive behaviour. McKinstry and Anil (2004) studied the consequences of repeated stunning of pigs. The animals in the experiment were stunned with a 50 Hz and 220 V electric current. The stunning was repeated within 15, 60 and 180 seconds after the first stunning. The following parameters were measured: time of spontaneous physical activity, time from stunning to regaining reflexes and time of epileptic activity. It was confirmed that repeated electric stunning may cause gradual epileptic activity and effective stunning did not depend on the time gap between the two stunning times. The tonic and clonic activity phase after the second stunning was significantly shorter than after only one electric stunning. The tendency to regain reflexes faster after double stunning was observed. The repeated stunning does not upgrade the welfare and should be used only when absolutely necessary (Council Regulation, 2009).

Scientific research has shown that the electric stunning of pigs causes more effusions and intensive haemorrhages in meat whereas gas stunning with CO_2 lowers the risk of PSE meat (Gregory, 2005). In the comparative study of fatteners, the progeny of Yorkshire × Landrace sows and Pietrain boars, stunned electrically and with CO_2 it was found that gas stunning causes higher calcium, potassium and glucose levels as well as stronger acidification and higher haematocrit values compared to electric stunning and compared to a controlled blood analysis performed on alive fatteners (Table 4). Such a condition occurs within seconds before anaesthesia.

In general the pH in the muscles of pigs stunned electrically lowers faster than in the muscles of pigs stunned with CO_2. The water holding capacity of meat is worse after electrical stunning even though the pH_{24} is the same in both stunning methods (Borzuta et al., 2018). The faster energetic metabolism after slaughter is caused by more intense muscle activity after electric stunning and more intense catecholamines secretion to the blood. More PSE carcasses occur after electric stunning e.g. Velarde et al. (2000) proved that in 4 experimental slaughterhouses the level of PSE carcasses obtained from pigs stunned with gas was 13.8% whereas with electric stunning it was 18.8% of PSE carcasses.

Table 4. Mean and standard error of energetic metabolism, acid-base balance and blood gases from stunned swine (Becerril-Herrera et al., 2009)

Variables	Baseline levels n = 159	Stunning with CO_2 n = 247	Stunning electrically n = 252
Blood pH	7.43±0.006 a	6.93±0.006 c	7.14±0.007 b
PCO_2 (mm Hg)	58.03±0.42 b	96.39±0.93 a	53.04±0.63 c
PO_2 (mm Hg)	32.02±0.57 a	27.55±0.58 b	27.48±0.52 b
Na^+ (mmol/ L)	141.57±0.17 b	140.64±0.67 b	146.13±0.47 a
K^+ (mmol/L)	5.40±0.03 c	14.20±0.14 a	9.91±0.13 b
Ca^{++} (mmol/ L)	1.27±0.006 c	1.45±0.006 a	1.29±0.005 b
Glucose (mg/dL)	76.57±0.44 c	201.49±4.41 a	184.98±3.55 b
Lactate (mg/ dL)	33.10±0.45 c	129.49±0.57 a	124.67±0.87 b
Haematocrit (%)	30.30±0.37 c	51.67±0.38 a	44.35±0.32 b

a, b, c – Different letters in the same row are statistically different, Tukey ($P \leq 0.05$).
n – number of observed pigs; SEM – standard error of the mean.

Lammens et al. (2006) when studying the electrical stunning of pigs effects with a triple electrode Midas device confirmed that the frequency of blood effusions in ham depended on sex, body weight and lean meat content. Effusions were observed in 23.19% of gilts' carcasses and only in 7.52% of castrated carcasses. Less effusions were observed in heavy carcasses (8.67%) than in light carcasses (19.41%). In carcasses with high lean meat content i.e. 61 to 67.8% the effusions occurred more often than in carcasses below 57.5% of the lean meat content reaching 21.31% and 7.98% respectively.

Different CO_2 concentration levels were tested in the gas tunnel (80% and 90%) as well as the different time of exposition of pigs to gas (70 or 100 seconds). The observations proved that stunning at 80% CO_2 for 70 and 100 seconds always resulted in stress causing high lactic acid level and lower pH than stunning with 90% CO_2 even when the time from stunning to sticking was longer i.e. 40–50 s instead of 25–35 s (Nowak et al., 2007). The Belgian authors (Van de Perre et al., 2010) confirmed these results.

On the other hand the Danish research showed that haemorrhages may occur in tenderloin and ham (*m. semimembranosus*) from pigs stunned with gas (Jorgensen et al., 2016, 2017). It is assumed that haemorrhages in tenderloin are the effect of broken lumbar vertebra because this muscle adjoins these bones.

The effect of CO_2 stunning on meat quality was also tested in cattle (Kim et al., 2013). One group of bullocks was stunned using a CO_2 mixture within 140 s and the other group was stunned with a gun. The gas stunning caused lower pH, lighter colour and longer sarcomere, improved meat tenderness and increased the drip loss in *m. longissimus thoracis* compared to the gun stunning.

Malaysian studies compared 3 stunning methods for heifers (mechanical, gun shooting and gun shooting with spinal cord penetration) with slaughter without stunning (the Halal method). It was reported that the quality of meat after all of these stunning methods is comparable and none of them could be recognized as being

a better method (Sazili et al., 2013). Similar studies on bulls were performed in Turkey (Önenç and Kaya, 2004). It was found that electric and shooting stunning caused higher glycogen levels in meat compared to the Turkish slaughter method without stunning. The studied methods did not have any effect on meat pH and water holding capacity but the sensory tests (flavour, colour, tenderness) showed that the meat after a gun shot was better than meat after electric stunning "head only" or without stunning.

The effect of different stunning methods was also studied on small ruminants. In Spanish studies the meat quality of Spanish Manchega lambs slaughtered using gas stunning and slaughtered without stunning was compared within the pH value, L*a*b* colour components, water holding capacity, thermal loss, shear force and drip loss in *m. longissimus dorsi* (Linares et al., 2007). After 24 h postmortem no significant differences in meat quality between the two groups were found. However, after 7 days of storage the pH, thermal losses and drip loss were significantly lower in the group slaughtered without stunning.

The study on goats treated with light anaesthesia by halothane intravenous injection and slaughtered without anaesthesia showed that meat from the group without anaesthesia was of higher glucose and lactic acid content (Sabow et al., 2016). Slaughter without stunning causes changes in the electroencephalographic activity (EEC) and might be regarded as stress when expecting pain. The catecholamine i.e. adrenaline and noradrenaline level tripled at slaughter in both slaughter methods and no significant differences between the two groups were observed.

Death by bleeding

According to medical science most of the countries define death as individual brain death (Terlouw et al., 2016 a). Depending on the country this definition includes the brain stem or the whole brain. Brain death is a break in function of the cerebral parenchyma structures responsible for the following life functions: breathing, thermoregulation and blood circulation. These structures are located in the brain stem. Even when the brain is dead there might still be some body movements caused by the residual activity of the nerves in the spinal cord (Laureys, 2005).

The goal of the bleeding process is to cause massive bleeding in order to slaughter the animal and to obtain the proper quality meat. In the bleeding process approx. 40–75% of all blood is removed (Tereszkiewicz and Choroszy, 2017). The main destructive effect of bleeding is in the internal organs, hypoxia especially brain and heart hypoxia is caused. In pigs the electroencephalographic changes in the brain can be observed within 36 seconds after the blood circulation has ended. Such a rapid effect of bleeding on the functioning of the brain is due to its low ability to store glucose and oxygen and its higher than other organs demand for these components. The experiment in this study proved that a break in the blood circulation stops brain activity within 12 to 72 seconds (depending on the species and glycogen supply). So the conclusion is that animals slaughtered ritually without stunning also lose consciousness. Sheep lose consciousness within 14 seconds or even quicker whereas cattle slaughtered without stunning lose consciousness within 7.5 to 75 seconds after sticking (Gregory et al., 2010). It was observed that 90% of slaughtered cattle fall down after 34 seconds or even sooner from the beginning of bleeding.

In the ritual slaughter unconsciousness takes place after sticking and by the bleeding process. The EU regulation states that during slaughter of animals stunning must be done first and the bleeding process afterwards. However, in respect of the religious denominations, Council Regulation No 93/119/WE allows desisting from stunning, according to religious ceremony.

The technique of sticking is mainly influencing the time needed to lose consciousness by the animal. Cutting the blood vessels on the level of the first cervical vertebra increases the intensity of bleeding and is more intense than in case of cutting the blood vessels on the level of the second or the third cervical vertebra, because the blood arteries on the level of further vertebrates are more difficult to reach (Gibson et al., 2015). Improving the bleeding technique and reversible stunning without damaging the heart is acceptable to many Muslims as the halal ritual slaughter (Nakyinsige et al., 2013). The stunning method called "head only" applied to sheep, goats and cattle is a good example. It is crucial to stick the animal as quickly as possible after stunning. The level of bleeding depends on heart activity. In many species irregular heart contractions may appear up to 5–10 minutes from the end of the bleeding no matter if the animals were stunned or not.

A very important goal of bleeding is to reach the blood flow that ensures meat durability. Bleeding is regarded as proper when 50% of blood is removed (Tereszkiewicz and Choroszy, 2017). According to Szkucik et al. (2001) bleeding can be divided into 5 levels: full bleeding i.e. 100% of blood is removed, followed by 75%, 50% and 25% of blood is removed and lack of bleeding that happens e.g. in dummy slaughter. The authors reported that in the vast majority of slaughtered animals the blood is fully removed and only few carcasses presented less than 75% of blood removal. According to Hariss (1984) in muscles there remain 2 to 9 ml of blood per kg of muscle. Such wide variability in blood residues depends not only on the level of carcass bleeding but also on the studied muscles. Szkucik et al. (2001) regards the muscle *obliquus internum abdominis* and the neck muscles (*m. colli*) as the most suitable muscles to determine the level of carcass bleeding. Less suitable are *longissimus dorsi* (poor blood supply) and diaphragm muscle (strong blood supply).

Carcass bleeding after slaughter is crucial for sanitary and hygienic parameters of slaughter raw materials (Tereszkiewicz et al., 2013). Proper carcass bleeding should be abundant and full and performed as soon as possible after stunning. Carcasses with blood are regarded as unfit for consumption whereas carcasses with not fully removed blood are regarded as less valuable. The blood pH is slightly alkaline (7.3 according to Tereszkiewicz et al., 2013) and prone to buffer. Blood is a good nourishment for microorganisms. The research proved clear relation between the level of carcass bleeding and microbiological quality and meat shelf life (Prost, 1985; Szkucik, 2000; Troeger et al., 2005). Meat from carcasses not fully bled is of limited technological usefulness especially as culinary meat and raw-maturing processed meat.

There is also the opinion that excessive blood removal has a negative effect on nutritional value and organoleptic meat features (Praca zbiorowa, 2011). The blood left in vessels is of very similar chemical composition to meat. This remaining blood

enhances slaughter efficiency, improves nutritional value as well as meat juiciness and palatability. There is a method of cattle slaughter without bleeding used in practice. The cattle chest is opened after stunning, the breathing stops and animal dies of suffocation. The meat obtained using this slaughter method, called the bloody steaks, is very juicy but not stable and the offal is unfit for consumption. This method is not included in the EU regulation and is used only incidentally in some countries e.g. in the USA.

Conclusions

To sum up the above presented review on stunning animals before slaughter it should be underlined that knowledge in this matter is comprehensive in the range of stunning techniques and consciousness indicators. Despite many stunning methods it is difficult to point to a method that would be similar to anaesthesia. It is necessary to continue research on the neurophysiological mechanisms of losing consciousness especially on eliminating pain. It would be helpful to broaden the understanding of the function of neurotransmitters and the use of magnetic resonance in the studies. It is crucial to eliminate instances of double stunning completely. Double stunning still occurs in practice in the case of manual stunning methods, especially in the electric method "head only" and in the shooting method. Technical improvement within these methods is required especially in placing and immobilizing the head at the moment of putting electrodes or the gun against the head. The results of studies on stunning methods effect on meat quality are not clear because some of them indicate and confirm this influence and others deny it. It is undoubtedly the consequence of high variability in the factors determining meat quality.

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